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Baseline and long-term gamma-glutamyltransferase, heart failure and cardiac arrhythmias in middle-aged Finnish men: prospective study and pooled analysis of published evidence

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Word count [5000]

Abstract

Purpose: To assess the associations of baseline and long-term GGT activity with risk of heart failure (HF), ventricular arrhythmias (VAs) and atrial fibrillation (AF).

Methods: GGT measurements were made in a prospective cohort of 1,780 men free of HF and cardiac arrhythmias at baseline. Correction for within-person variability was made using data from repeat measurements taken several years apart.

Results: During an average follow-up of 22 years, 222 HFs, 56 VAs, and 336 AFs events occurred. The regression dilution ratio of $\log_e$ GGT was 0.68 [95% confidence interval (CI): 0.61-0.74]. Serum GGT was log-linearly associated with risk of HF, VAs, and AF. In analyses adjusted for established risk factors, the hazard ratios (HRs) (95% CIs) for HF, VAs, and AF per 1 standard deviation (SD) higher baseline $\log_e$ GGT values were 1.25 (1.07-1.45), 1.37 (1.04-1.80), and 1.04 (0.92-1.18) respectively. After correction for within-person variability, the corresponding HRs were 1.38 (1.11-1.73), 1.58 (1.06-2.37), and 1.06 (0.88-1.27). These findings remained consistent in analyses accounting for incident coronary heart disease and the development of impaired renal function. In a meta-analysis of five population-based studies, the fully-adjusted relative risks for HF per 1 SD higher baseline and long-term GGT values were 1.28 (1.20 to 1.35) and 1.43 (1.31-1.56) respectively. In pooled analysis of two studies, the corresponding risks for AF were1.09 (1.02-1.16) and 1.14 (1.03-1.25) respectively.

Conclusion: GGT is positively and log-linearly associated with future risk of HF, VAs, and AF. Further research is needed to assess the causal relevance of these findings.

Keywords

Gamma-glutamyltranferase; heart failure; ventricular arrhythmias; atrial fibrillation

Abstract word count [249]

Introduction

Gamma-glutamyltransferase (GGT) activity has routinely been used in clinical practice to help indicate potential hepatic or biliary disease.[1] Elevated GGT values may also reflect accumulation of hepatic fat,[2] oxidative stress,[3] and have been shown to be associated with cardiovascular disease (CVD) outcomes. Several prospective studies have consistently demonstrated associations between GGT and risk of coronary heart disease (CHD),[4] stroke,[4] CVD mortality,[5] and composite CVD events.[4, 6] However, uncertainty persists regarding the association of GGT with HF and cardiac arrhythmias [(ventricular arrhythmias (VAs) and atrial fibrillation (AF)]. Heart failure is associated with unacceptably high morbidity and mortality risks[7] and imposes a significant economic burden on health systems. Cardiac rhythm disturbances, with AF being the most commonly diagnosed arrhythmia in clinical practice, are considered to be the final events in a chain of complications leading to stroke and increased overall mortality.[8] Cardiac arrhythmias and HF often coexist and share many common risk factors such as older age, hypertension, myocardial infarction, and diabetes.[9] Both VAs and AF are associated with an increased risk of HF events and vice versa, with death being a common consequence.[10] Though a number of population-based prospective studies have shown a positive association between baseline GGT activity and risk of HF events,[5, 11, 12] no study has at yet assessed the long-term relevance of GGT activity to HF. There is very little information about the extent to which GGT fluctuates within individuals, as these data enhances the interpretation of epidemiological studies with an aetiological motivation. We have recently shown that GGT exhibits high within-person variability,[13] which could be the result of measurement errors in assays, fluctuations due to acute phase reactions, lifestyle changes, ageing, and chronic disease. Therefore, analysis using only baseline measurements of GGT could underestimate the true strength of any aetiological association between GGT and disease outcome (i.e. "regression dilution bias"[14]). Previous studies may have considerably under-estimated the association between GGT and HF, therefore a need to estimate and correct for the effect of this regression dilution bias. Prospective data regarding the association between GGT and AF are sparse; Alonso and colleagues in the Atherosclerosis Risk in

Communities Study (ARIC) reported an association between GGT activity and an increased risk of AF.[15] To our knowledge, the prospective association between GGT and risk of VAs has however not been previously investigated.

Against this background, we aimed to quantify more reliably than previously possible, the associations of GGT with risk of HF, VAs, and AF in a population-based cohort of 1,780 apparently healthy men from eastern Finland. To put our results into context, we also performed pooled analyses of available published prospective evidence on the associations.

Methods

Details of study population, endpoint ascertainment, risk factor assessment, statistical analyses, and literature search strategy can be found in **Supplementary Materials 1 and 2**.

Results

Baseline characteristics and correlates of gamma-glutamyltransferase

Table 1 summarizes baseline characteristics of the 1,780 participants in the KIHD study. The mean age of the participants was 53 (SD 5) years. Median (interquartile range) GGT value was 20 (15-32) U/L. During an average follow-up of 22 years, there were 222 HF events (annual rate 5.67/1,000 person-years at risk; 95% CI: 4.97 to 6.47); 56 incident VAs (annual rate 1.42/1,000 person-years at risk; 95% CI: 1.09 to 1.84); and 336 incident AF events (annual rate 8.93/1,000 person-years at risk; 95% CI: 8.03 to 9.94). Serum GGT values were weakly to moderately and positively correlated with physical measures (BMI, blood pressure, and resting heart rate) and with several lipid, metabolic, and inflammatory markers. Weak inverse correlations were observed for age (r = -0.03) and HDL-C (r = -0.04). Baseline GGT values were higher in men with diabetes compared with men without diabetes, higher in men with a history of hypertension compared with men without a history of hypertension, and higher in current smokers compared with non-smokers.

Correction for within-person variability

Repeat measurements of GGT taken 4 years and 11 years after baseline were available in a random sample of 624 men, yielding a total of 1,143 repeat measurements of GGT. Overall, the regression dilution ratio (RDR) of loge GGT, adjusted for age, was 0.68 (95% CI: 0.61to 0.74), suggesting that the associations using one-off or baseline measurements of GGT with the outcomes could be under-estimated by [(1/0.68)-1]*100 = 47%.

GGT and risk of heart failure

Prospective cohort analysis

In analyses adjusted for conventional risk factors (age, BMI, SBP, prevalent coronary heart disease, smoking status, history of diabetes mellitus, LVH, use of antihypertensive agents and lipid-lowering drugs), there was a log-linear association between GGT and HF risk (**Figure 1; Supplementary Materials 3 and 4**). The age-adjusted HR per 1 SD change in $\log_e$ GGT value was 1.45 (95% CI: 1.28 to 1.67; $P < 0.001$), which was somewhat attenuated following further adjustment for established risk factors 1.25 (95% CI: 1.07 to 1.45; $P = 0.004$). After correction for within-person variability in GGT values, the similarly adjusted HRs were 1.74 (95% CI: 1.43 to 2.12; $P < 0.001$) and 1.38 (95% CI: 1.11 to 1.73; $P = 0.004$) respectively. The results remained consistent on further adjustment for alcohol consumption, resting heart rate, triglycerides, total cholesterol, HDL-C, eGFR, and CRP and remained unchanged after further adjusting for incident CHD events during follow-up (253 cases) (**Table 2)**. In adjustment for the development of impaired renal function during follow-up (172 cases), the results remained similar 1.23 (95% CI: 1.05 to 1.44; $P = 0.011$) and 1.36 (95% CI: 1.07 to 1.72; $P = 0.011$) per 1 SD higher baseline and usual $\log_e$ GGT values respectively. The total number of deaths that occurred during follow-up was 814, of which 365 were CVD deaths. In analyses including death as a competing risk event, the HRs were 0.96 (95% CI: 0.74 to 1.25; $P = 0.769$) and 0.94 (95% CI: 0.64 to 1.38; $P = 0.769$) per 1 SD change in baseline

and usual $\log_e$ GGT values respectively. HRs did not vary importantly by levels or categories of pre-specified conventional risk factors (*P* for interaction > 0.05 for each) (**Supplementary Material 5**), and the main results remained similar in analyses that excluded the first five years of follow-up, participants with GGT values greater than three times the upper limit of normal, and participants with potential fatty liver disease (Data not shown). In subsidiary analyses, we found significant evidence of associations of GGT with CVD mortality and nonfatal HF (**Supplementary Materials 6 and 7**).

Literature-based meta-analysis

Including the current study, we identified five population-based prospective studies[5, 11, 12, 16] reporting on the association between GGT and HF risk (**Supplementary Materials 8 and 9**). In a pooled analysis of 210,841 participants and 1,821 HF events, the RRs for HF per 1 SD higher baseline and usual GGT values, typically adjusted for several conventional and emerging risk factors were 1.28 (95% CI: 1.20 to 1.35; $P < 0.001$) and 1.43 (95% CI: 1.31 to 1.56; $P < 0.001$) respectively (**Figure 2**). The summary RR was identical when a fixed effect model was employed. Exclusion of any single study at a time from the pooled analysis had minimal effect on the pooled RR. We found no evidence of heterogeneity among the contributing studies (I^2=0%; P=0.791).

Association of GGT with risk of cardiac arrhythmias

In analyses adjusted for conventional risk factors, there was an approximately log-linear association between GGT and VAs (**Figure 1; Supplementary Materials 3 and 4**). The age-adjusted HR for VAs per 1 SD change in baseline $\log_e$ GGT value was 1.48 (95% CI: 1.16 to 1.90; $P = 0.002$), which was minimally attenuated following further adjustment for established risk factors 1.37 (95% CI: 1.04 to 1.80; $P = 0.026$). Similarly adjusted HRs per 1 SD change in usual $\log_e$ GGT values were1.78 (95% CI: 1.24 to 2.56; $P = 0.002$) and 1.58 (95% CI: 1.06 to 2.37; $P = 0.026$) respectively. The results were somewhat attenuated on further adjustment for alcohol consumption, resting heart rate, triglycerides, total

cholesterol, HDL-C, and eGFR and remained consistent on further adjustment for CRP and accounting for incident coronary events (**Table 2**). Hazard ratios did not vary importantly by levels or categories of pre-specified conventional risk factors (*P* for interaction > 0.10 for each) (**Supplementary Material 10**). Whereas, there was a log-linear positive association of GGT with AF risk in analyses initially adjusted for age, the association was less robust on further adjustment for conventional risk factors (**Figure 1; Supplementary Materials 3 and 4; Table 2**). In pooled analysis of the KIHD and ARIC studies (11,113 participants and 1,357 AF cases), the fully adjusted HRs for AF per 1 SD higher baseline and usual GGT values were 1.09 (95% CI: 1.02 to 1.16; *P* = 0.008) and 1.14 (95% CI: 1.03 to 1.25; *P* = 0.008).

Discussion

In addition to assessing the shape and magnitude of the prospective associations of baseline GGT activity with HF and AF, our study is the first to evaluate these aspects of the association of GGT with risk of VAs. By correcting for regression dilution using repeat measurement of GGT, we have also shown that these associations are considerably under-estimated (by approximately half) when baseline measurements of GGT are used. In this population of middle-aged men without HF and history of cardiac arrhythmias at baseline, whereas GGT was positively and log-linearly associated with risk of HF and VAs in analyses adjusted for several conventional risk factors; the initial positive log-linear association of GGT with risk of AF in age-adjusted analysis was somewhat attenuated on further adjustment for conventional risk factors. Alonso and colleagues in the ARIC study have also demonstrated a linear relationship to the association between GGT and AF, but their association remained independent of several potential confounders including incident coronary artery disease events as a time-dependent covariate.[15] However, in our pooled analysis of the two studies, there was a statistically significant association between GGT and AF.

Our findings remained consistent across several subgroups and levels of cardiovascular risk markers, and were unchanged in analyses that adjusted for CHD and impaired renal function during follow-up and in several sensitivity analyses. Pooled findings from the meta-analysis of five studies reinforces the

validity and generalizability of the GGT-HF association, suggesting that a two fold increase in usual GGT values is associated with approximately 40% higher risk of HF. However, given the high mortality rate in our study cohort which might have hindered our event of interest, the association between GGT and HF was attenuated when death was adjusted for as a competing risk event. This was not a surprising finding, as total deaths in the cohort included CVD mortality events and our subsidiary analysis demonstrated GGT to be associated with CVD mortality.

Possible explanations for findings

Proposed mechanistic pathways underpinning the associations of elevated GGT values and increased risk of HF and cardiac arrhythmias, include the pro-oxidant and pro-inflammatory activities[17] of GGT and its direct involvement in atheromatous plaque formation.[18] Other pathways implicated include underlying fatty liver,[17] which is associated with low-grade inflammation, insulin resistance, and oxidative stress,[19, 20] - all known to be associated with increased risk of these cardiovascular outcomes.[21-23] Endothelial dysfunction and exposure to environmental pollutants have also been postulated.[24, 25] Given that CHD is a major cause of HF[26] and the consistent observational association demonstrated between GGT and CHD,[27] underlying CHD may be mediating the association between GGT and HF. However, our association between GGT and HF remained persistent on accounting for incident CHD during follow-up. There is also a possibility that the association between GGT and HF may be due to reverse causation, as GGT is frequently increased in HF due to liver dysfunction commonly encountered in HF.[28] However, this is unlikely given (i) that our analysis was pre-specified to include participants without a history of HF at baseline; (ii) the mean follow-up period (> 20 years) was sufficiently long to ascertain the risk for HF; and (iii) the findings remained robust on excluding the first five years of follow-up. In addition, given that GGT is also synthesized by the kidneys, there is a close relationship with renal function; therefore the association of GGT with HF may also reflect impaired renal function.[29] Our findings, however, remained largely unchanged after adjusting for baseline renal function and accounting for the development of

impaired renal function. The robust linear and independent relationships demonstrated are suggestive of causality, but these require confirmation in robust randomized controlled trials. There are several pharmacological agents (e.g. insulin sensitizers and antioxidants) that modify levels of GGT, however, they also alter levels of other liver enzymes and lipid factors.[30] In the absence of clinical trials however, Mendelian randomisation (MR) studies of genetic variants specifically related to GGT levels may provide another route to assess causality.[31]

Implications of findings

Our findings are relevant and may have clinical implications. It further extends the evidence base and thus highlights a clear link between serum GGT and the development of cardiovascular outcomes. The findings underscore a potentially deleterious role of increasing GGT activity on future risk of a wide range of adverse cardiovascular outcomes in the general population. Given that assays for GGT are sensitive, well standardised, simple, quick, inexpensive, commonly measured as part of routine liver function panels, and do not require a fasting state prior to venepuncture; they have the potential to be used in the identification of individuals at high risk of these adverse cardiovascular outcomes and in developing treatment methods. However, additional research is needed to help discover the mechanistic pathways of GGT in the pathogenesis of cardiovascular outcomes and larger studies are needed to confirm these findings.

Strengths and limitations

The notable strengths include a large sample that was selected to be a nationally representative population-based sample of middle-aged men, was well characterised, involved high response and there were no losses during follow-up, reducing the risk of selection bias. Participants have been prospectively monitored using established databases for hospital admissions, supplemented with reliable data on a comprehensive panel of lifestyle and biological markers, including medication for hypertension and dyslipidaemia, to allow adequate adjustment for potential confounding; enabling reliable and independent

assessments of the associations. The mean follow-up period was sufficiently long to ascertain the risk for the various endpoints in the general population. Repeat measurements of GGT made within the same individuals over time were available, enabling correction for within-person variability. Several sensitivity analyses were conducted to ensure the robustness of our results. To put our GGT-HF results into context, we conducted a meta-analysis of five studies (including the present study) and demonstrated the reliability of our cohort analyses. The limitations of the present study also deserve mention. We were unable to assess the differential impact of GGT on risk of HF with preserved versus reduced ejection fractions, because there were no data on ventricular function post-HF development. There was no detailed information on whether cardiac arrhythmic events were paroxysmal (or treated by cardioversion) because arrhythmias were based on hospitalisation data. However, we detected the clinically most important arrhythmic events. There is a possibility that very short events of VAs and AF might have might have been missed without continuous heart rhythm monitoring systems in place. In the KIHD study, majority of sudden cardiac deaths (SCDs) that occurred during follow-up, occurred out-of-hospital (~80%)[13]; therefore it was not possible to detect all cases of VAs, given that SCD is generally considered to be a complication of ventricular fibrillation/tachycardia. In addition, ascertainment of arrhythmic events relied a lot on hospitalisation discharge codes, potentially missing cases that were asymptomatic. However, the validity of this approach for epidemiologic studies of this nature has been demonstrated to be adequate.[32, 33] Though a comprehensive panel of confounders was taken into account to ensure the validity of our results, potential residual confounding due to other unmeasured confounders (such as antioxidants, and other medications such as phenytoin or barbiturates that affect baseline levels of GGT) cannot be entirely ruled out. Measurements of other liver function enzymes such as the aminotransferases, were not made in the KIHD study, preventing comparison of the separate and joint associations of different liver function enzymes with the outcomes assessed. The study included only middle-aged Finnish men from eastern Finland and cannot necessarily be extrapolated to the young, women and other populations. However, the

pooled analyses for the GGT-HF and GGT-AF associations involved studies conducted in both men and women.

Conclusions

GGT activity is positively and log-linearly associated with future risk of HF, VAs, and AF. Further research is needed to assess the causal relevance of these findings.

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Declaration of Conflicting Interests

The authors declare that there is no conflict of interest.

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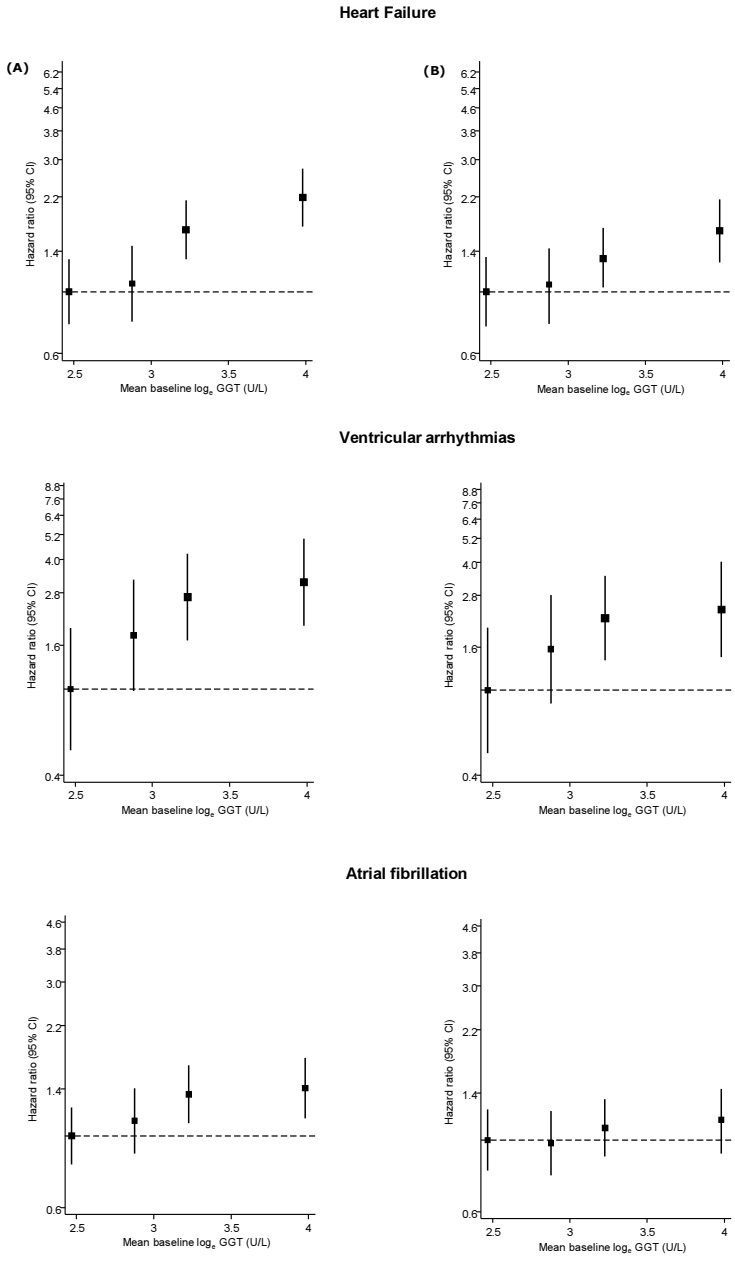
Figure 1. Hazard ratios for heart failure, ventricular arrhythmias, and atrial fibrillation, by quartiles of baseline values of $\log_e$ GGT



A, adjusted for age; **B**, adjusted for age, body mass index, systolic blood pressure, prevalent coronary heart disease, smoking status, history of diabetes, left ventricular hypertrophy, and use of medications (antihypertensive agents and lipid-lowering drugs); GGT, gamma-glutamyltransferase; the mean GGT

value (U/L) was 12.1 for the lowest quartile; 17.9 for the second quartile; 25.5 for the third quartile; and 44.3 for the top quartile.

Figure 2. Relative risks for heart failure per 1 SD higher baseline and usual distribution of GGT values in published prospective studies

Study acronyms are provided in **Supplementary Material 7**; **A**, relative risks per 1 standard deviation (SD) higher baseline GGT values; **B**, relative risks per 1 SD higher usual GGT values; Size of data markers are proportional to the inverse of the variance of the relative risk; CI, confidence interval (bars); GGT, gamma-glutamyltransferase; HF, heart failure

Table 1. Baseline characteristics and cross-sectional correlates of gamma-glutamyltransferase

	Mean (SD) or %	Pearson correlation r (95% CI)†	Percentage difference (95% CI) in GGT values per 1 SD higher or compared to reference category of correlate‡
Log_e GGT (U/L)	3.11 (0.63)		
Questionnaire/Prevalent conditions			
Age at survey (yrs)	52.6 (5.0)	-0.03 (-0.08, 0.02)	-2% (-5, 1)
Alcohol consumption (g/week)	76.1 (140.2)	0.27 (0.23, 0.31)***	19% (15, 22)***
History of diabetes			
No	95.4	-	Ref
Yes	4.6	-	47% (28, 69)***
Smoking status			
Other	67.6	-	Ref
Current	32.4	-	11% (4, 18)**
Left ventricular hypertrophy			
No	98.9	-	Ref
Yes	1.1	-	-4% (-28, 28)
History of hypertension			
No	71.0	-	Ref
Yes	29.0	-	27% (20, 36)***
History of CHD			
No	78.1	-	Ref
Yes	21.9	-	17% (9, 25)***
Use of anti-hypertensives			
No	81.7	-	Ref
Yes	18.3	-	24% (15, 34)***
Medication for dyslipidemia			
No	99.4	-	Ref
Yes	0.6	-	25% (-14, 81)
Physical measurements			
BMI (kg/m^2)	26.8 (3.5)	0.34 (0.30, 0.38)***	24% (21, 27)***
SBP (mmHg)	133.9 (16.6)	0.21 (0.17, 0.26)***	14% (11, 18)***
DBP (mmHg)	89.0 (10.5)	0.25 (0.21, 0.30)***	17% (14, 21)***
Resting heart rate (bpm)	62.5 (10.7)	0.14 (0.10, 0.19)***	9% (6, 13)***
Lipid markers			
Total cholesterol (mmol/l)	5.93 (1.10)	0.10 (0.05, 0.14)***	6% (3, 9)***
HDL-C (mmol/l)	1.29 (0.30)	-0.04 (-0.09, 0.01)	-2% (-5, 0)
Log_e triglycerides (mmol/l)	0.09 (0.50)	0.26 (0.21, 0.30)***	18% (14, 21)***
Metabolic, inflammatory, and renal markers			
Fasting plasma glucose (mmol/l)	5.33 (1.21)	0.21 (0.16, 0.25)***	14% (11, 17)***
Serum creatinine (µmol/1)	89.4 (22.5)	0.00 (-0.04, 0.05)	0% (-3, 3)
Log_e C-reactive protein (mg/l)	0.29 (0.96)	0.27 (0.22, 0.31)***	18% (15, 21)***
Estimated GFR (ml/min/1.73 m^2)	87.7 (17.5)	-0.01 (-0.05, 0.04)	-0% (-3, 3)

BMI, body mass index; CHD, coronary heart disease; DBP, diastolic blood pressure; HDL-C, high-density lipoprotein cholesterol; GGT, gamma-glutamyltransferase; SD, standard deviation; SBP, systolic blood pressure; ‡Percentage change in GGT values per 1-SD increase in the row variable (or for categorical variables, the percentage difference in mean GGT values for the category versus the reference) adjusted for age; asterisks indicate the level of statistical significance: *P<0.05, **P<0.01, ***P<0.001

Table 2. Associations of baseline and usual gamma-glutamyltransferase values with heart failure, ventricular arrhythmias, and atrial fibrillation

Models	Heart failure		Ventricular arrhythmias		Atrial fibrillation	
	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value	Hazard ratio (95% CI)	*P*-value
	1,780 participants and 222 cases		1,780 participants and 56 cases		1,780 participants and 336 cases	
			Baseline GGT			
Model 1	1.45 (1.28 to 1.67)	< 0.001	1.48 (1.16 to 1.90)	0.002	1.15 (1.03 to 1.29)	0.011
Model 2	1.25 (1.07 to 1.45)	0.004	1.37 (1.04 to 1.80)	0.026	1.04 (0.92 to 1.18)	0.522
Model 3	1.26 (1.07 to 1.47)	0.004	1.30 (0.98 to 1.75)	0.068	1.05 (0.92 to 1.19)	0.502
Model 4	1.24 (1.05 to 1.45)	0.009	1.31 (0.98 to 1.76)	0.069	1.06 (0.93 to 1.21)	0.412
Model 5	1.25 (1.07 to 1.46)	0.006	1.32 (0.99 to 1.77)	0.062	1.06 (0.93 to 1.21)	0.405
			Usual GGT			
Model 1	1.74 (1.43 to 2.12)	< 0.001	1.78 (1.24 to 2.56)	0.002	1.24 (1.05 to 1.46)	0.011
Model 2	1.38 (1.11 to 1.73)	0.004	1.58 (1.06 to 2.37)	0.026	1.06 (0.88 to 1.27)	0.522
Model 3	1.40 (1.11 to 1.77)	0.004	1.49 (0.97 to 2.28)	0.068	1.07 (0.89 to 1.30)	0.502
Model 4	1.36 (1.08 to 1.72)	0.009	1.49 (0.97 to 2.30)	0.069	1.09 (0.90 to 1.32)	0.412
Model 5	1.39 (1.10 to 1.75)	0.006	1.51 (0.98 to 2.32)	0.062	1.09 (0.90 to 1.32)	0.405

GGT, gamma-glutamyltransferase; hazard ratios are reported per 1 standard deviation increase in $\log_e$ GGT levels; 1 standard deviation higher $\log_e$ GGT was approximately equivalent to two-fold higher GGT values.
Model 1: Adjusted for age
Model 2: Model 1 plus BMI, systolic blood pressure, prevalent coronary heart disease, smoking status, history of diabetes, left ventricular hypertrophy, and use of medications (antihypertensive agents and lipid-lowering drugs)
Model 3: Model 2 plus alcohol consumption, resting heart rate, triglycerides, total cholesterol, high-density lipoprotein cholesterol, and estimated glomerular filtration rate
Model 4: Model 3 plus C-reactive protein
Model 5: Model 4 plus incident coronary heart disease as a time-dependent covariate

SUPPLEMENTARY MATERIAL

Supplementary Material 1	Study population, ascertainment of outcomes, and risk factor assessment
Supplementary Material 2	Systematic review and meta-analysis methodology
Supplementary Material 3	PRISMA checklist
Supplementary Material 4	MOOSE checklist
Supplementary Material 5	Literature search strategy
Supplementary Material 6	Hazard ratios for heart failure, ventricular arrhythmias, and atrial fibrillation using multivariate-adjusted fractional polynomials
Supplementary Material 7	Hazard ratios for baseline and usual $\log_e$ GGT values and heart failure risk by several participant level characteristics
Supplementary Material 8	Selection of studies included in the meta-analysis
Supplementary Material 9	Prospective studies of gamma-glutamyltransferase and incident heart failure
Supplementary Material 10	Hazard ratios for baseline and usual $\log_e$ GGT values and ventricular arrhythmias risk by several participant level characteristics

Supplementary Material 1: Study population, ascertainment of outcomes, and risk factor assessment

Study population

The study population consisted of a representative sample of men living in the city of Kuopio and its surrounding rural communities in eastern Finland. Subjects were participants in the Kuopio Ischaemic Heart Disease (KIHD) risk factor study, a longitudinal population-based study designed to investigate risk factors for CVD, atherosclerosis and related outcomes.[1] Participants were 42-61 years of age during baseline examinations performed between March 1984 and December 1989. Of 3,433 potentially randomly eligible and randomly selected men, 2,682 (78%) volunteered to participate; 186 did not respond to the invitation and 367 declined to give informed consent. Men with a prevalent history of HF, cardiac arrhythmias, or liver disease were excluded (n=198). The final cohort for the present analysis included 1,780 men with non-missing information on serum GGT and covariates. The KIHD study complies with the Declaration of Helsinki and the Research Ethics Committee of the University of Eastern Finland approved the study, and each participant gave written informed consent.

Ascertainment of outcomes

All outcomes (HF, VAs, and AF) that occurred from study enrollment through 2012 were included. There were no losses to follow-up. In the KIHD study, participants are under continuous surveillance for the development of new CVD events, including new incident HF, VAs, and AF events.[2] The sources of information on outcomes were based on a comprehensive review of hospital records and discharge diagnoses, inpatient physician claims data, study ECGs, and medico-legal reports. The diagnostic classification of HF cases was coded according to the ICD-10 codes (I50.0-I50.9, I11, I42.0-I42.9).). The diagnosis of HF was based on diagnostic guidelines of the European Society of Cardiology[3] and which included criteria such as symptoms, signs, laboratory investigations including the determination of natriuretic peptides, chest radiography results, echocardiography as well as electrocardiographic findings. The diagnostic classification of VAs was coded according to ICD-9 codes (427.41) or ICD-10 codes (I47.2, I49.0) codes. The definition of non-sustained or sustained ventricular tachycardia and/or ventricular fibrillation was based on electrocardiography.[4] The diagnostic classification of AF cases was coded according to ICD-10 codes (I48.0-I48.9).[5] Documents were cross-checked in detail by two physicians. The Independent Events Committee, masked to clinical data, performed classification of outcomes.

Measurement of risk factors

Collection of blood specimens and the measurement of serum lipids, lipoproteins and glucose have been described previously.[6] Blood samples were taken between 08:00 and 10:00 hours. In addition to fasting, participants were instructed to abstain from drinking alcohol for at least 3 days and from smoking for at least 12 h prior to assessment. The serum samples were stored frozen at -80 °C for 0.2-2.5 years. Fasting plasma glucose (FPG) was measured by the glucose dehydrogenase method (Merck, Darmstadt, Germany). Serum GGT activity was measured using the kinetic method (Thermo Fisher Scientific, Vantaa, Finland). Serial measurements of GGT were performed 4 years and 11 years apart during a 22 year period in a random subset of participants. C-reactive protein (CRP) was measured with an immunometric assay (Immulite High Sensitivity C-Reactive Protein Assay; DPC, Los Angeles, CA, USA). Smoking, alcohol consumption and blood pressure were assessed as described previously.[6] Body mass index (BMI) was computed as the ratio of weight in kilograms to the square of height in metres. Standard resting 12-lead ECG was also recorded. The ECG criterion for left ventricular hypertrophy (LVH) was based on either the Sokolow-Lyon or Romhilt-Estes point score.[7-10]

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1. Salonen JT. Is there a continuing need for longitudinal epidemiologic research? The Kuopio Ischaemic Heart Disease Risk Factor Study. *Ann Clin Res*. 1988; 20: 46-50.

2. Karppi J, Kurl S, Makikallio TH, Ronkainen K and Laukkanen JA. Serum beta-carotene concentrations and the risk of congestive heart failure in men: A population-based study. *Int J Cardiol*. 2013 Jan 17. pii: S0167-5273(12)01701-9. doi: 10.1016/j.ijcard.2012.12.072.

3. McMurray JJ, Adamopoulos S, Anker SD, et al. ESC Guidelines for the diagnosis and treatment of acute and chronic heart failure 2012: The Task Force for the Diagnosis and Treatment of Acute and Chronic Heart Failure 2012 of the European Society of Cardiology. Developed in collaboration with the Heart Failure Association (HFA) of the ESC. *European heart journal*. 2012; 33: 1787-847.

4. Zaccardi F, Webb DR, Kurl S, Khunti K, Davies MJ and Laukkanen JA. Inverse association between fasting plasma glucose and risk of ventricular arrhythmias. *Diabetologia*. 2015.

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6. Salonen JT, Nyyssonen K, Korpela H, Tuomilehto J, Seppanen R and Salonen R. High stored iron levels are associated with excess risk of myocardial infarction in eastern Finnish men. *Circulation*. 1992; 86: 803-11.

7. Sokolow M and Lyon TP. The ventricular complex in left ventricular hypertrophy as obtained by unipolar precordial and limb leads. 1949. *Ann Noninvasive Electrocardiol*. 2001; 6: 343-68.

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9. Oikarinen L, Karvonen M, Viitasalo M, et al. Electrocardiographic assessment of left ventricular hypertrophy with time-voltage QRS and QRST-wave areas. *Journal of human hypertension*. 2004; 18: 33-40.

10. Schillaci G, Battista F and Pucci G. A review of the role of electrocardiography in the diagnosis of left ventricular hypertrophy in hypertension. *J Electrocardiol*. 2012; 45: 617-23.

Supplementary Material 2: Systematic review and meta-analysis methodology

A systematic review was conducted using a predefined protocol and in accordance with the PRISMA and MOOSE guidelines[1, 2] (**Supplementary Materials 3-4**). Prospective (cohort or nested case-control) studies of the association between GGT and incident HF that were published up to November 2015 were sought using computer-based databases (MEDLINE, EMBASE, and Science Citation Index). We crossed the term 'gamma-glutamyltransferase' (and similar) with "heart failure", "left ventricular dysfunction" (and similar terms) without any language restrictions. Further details on the search strategy are presented in **Supplementary Material 5**. Reference lists of the retrieved articles were searched for additional articles. Studies were eligible for inclusion if they had at least one year of follow-up. The relative risk (RR) with 95% confidence intervals (CIs) was used as the common measure of association across studies. To enable a consistent approach to the meta-analysis and enhance consistency, reported study-specific RRs were transformed to per 1SD change in baseline GGT values using standard statistical methods [3, 4] which have been described in detail previously.[5, 6] Briefly, log risk estimates were transformed assuming a normal distribution (or that a transformation of the explanatory variable for which the risk ratio is based was normally distributed). The log risk ratio for a 1 SD change being equivalent to the log risk ratio for a comparison of extreme thirds divided by 2.18 (equivalently, as the log risk ratio for a comparison of extreme quarters divided by 2.54 or as the log risk ratio for a comparison of extreme quintiles divided by 2.80). Standard errors of the log risk estimates were calculated using published confidence limits and were standardized in the same way. Associations of usual levels of GGT and HF were estimated using the correction factor derived from the KIHD Study. The summary RR (including the estimate from the present study) was calculated using random effects meta-analysis (subsidiary analyses used a fixed effect meta-analysis). Statistical heterogeneity across studies was quantified using standard chi-square tests and the I^2 statistic.[7]

References

1. Moher D, Liberati A, Tetzlaff J and Altman DG. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *PLoS Med*. 2009;6:e1000097.

2. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, Moher D, Becker BJ, Sipe TA, Thacker SB and Group ftM-aOOSiE. Meta-analysis of Observational Studies in Epidemiology. *JAMA: The Journal of the American Medical Association*. 2000;283:2008-2012.

3. Chêne G and Thompson SG. Methods for Summarizing the Risk Associations of Quantitative Variables in Epidemiologic Studies in a Consistent Form. *American Journal of Epidemiology*. 1996;144:610-621.

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5. Kunutsor SK, Apekey TA and Khan H. Liver enzymes and risk of cardiovascular disease in the general population: A meta-analysis of prospective cohort studies. *Atherosclerosis*. 2014;236:7-17.

6. Kunutsor SK, Apekey TA, Hemelrijck MV, Calori G and Perseghin G. Gamma glutamyltransferase, alanine aminotransferase and risk of cancer: Systematic review and meta-analysis. *International journal of cancer Journal international du cancer*. 2014.

7. Higgins JP, Thompson SG, Deeks JJ and Altman DG. Measuring inconsistency in meta-analyses. *BMJ*. 2003;327:557-60.

Supplementary Material 3: PRISMA 2009 check-list

Section/topic	Item No	Checklist item	Reported on page No
Title			
Title	1	Identify the report as a systematic review, meta-analysis, or both	1
Abstract			
Structured summary	2	Provide a structured summary including, as applicable, background, objectives, data sources, study eligibility criteria, participants, interventions, study appraisal and synthesis methods, results, limitations, conclusions and implications of key findings, systematic review registration number	2
Introduction			
Rationale	3	Describe the rationale for the review in the context of what is already known	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS)	Methods
Methods			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (such as web address), and, if available, provide registration information including registration number	Methods
Eligibility criteria	6	Specify study characteristics (such as PICOS, length of follow-up) and report characteristics (such as years considered, language, publication status) used as criteria for eligibility, giving rationale	Methods
Information sources	7	Describe all information sources (such as databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched	Methods
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated	Supplementary Material 5
Study selection	9	State the process for selecting studies (that is, screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis)	Methods
Data collection process	10	Describe method of data extraction from reports (such as piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators	Methods
Data items	11	List and define all variables for which data were sought (such as PICOS, funding sources) and any assumptions and simplifications made	Methods
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis	Not applicable
Summary measures	13	State the principal summary measures (such as risk ratio, difference in means).	Methods
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (such as I^2 statistic) for each meta-analysis	Methods
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (such as publication bias, selective reporting within studies)	Not applicable
Additional analyses	16	Describe methods of additional analyses (such as sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-	Not applicable

Section/topic	Item No	Checklist item	Reported on page No
		specified	
Results			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram	Supplementary Material 8
Study characteristics	18	For each study, present characteristics for which data were extracted (such as study size, PICOS, follow-up period) and provide the citations	Supplementary Material 9
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome-level assessment (see item 12).	Not applicable
Results of individual studies	20	For all outcomes considered (benefits or harms), present for each study (a) simple summary data for each intervention group and (b) effect estimates and confidence intervals, ideally with a forest plot	Figure 3
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency	Figure 3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see item 15)	Not applicable
Additional analysis	23	Give results of additional analyses, if done (such as sensitivity or subgroup analyses, meta-regression) (see item 16)	Not applicable
Discussion			
Summary of evidence	24	Summarise the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (such as health care providers, users, and policy makers)	Discussion
Limitations	25	Discuss limitations at study and outcome level (such as risk of bias), and at review level (such as incomplete retrieval of identified research, reporting bias)	Discussion
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research	Discussion
Funding			
Funding	27	Describe sources of funding for the systematic review and other support (such as supply of data) and role of funders for the systematic review	None

Supplementary Material 4: MOOSE checklist

Baseline and long-term gamma-glutamyltransferase and risk of heart failure and cardiac arrhythmias: prospective study and meta-analysis

Criteria		Brief description of how the criteria were handled in the review
Reporting of background		
√	Problem definition	Elevated baseline circulating gamma-glutamyltransferase (GGT) has been demonstrated to be associated with risk of incident heart failure (HF), but the precise nature and magnitude of the association is uncertain
√	Hypothesis statement	There is a linear and positive relationship between GGT and HF risk
√	Description of study outcomes	Heart failure
√	Type of exposure	Serum measurements of GGT
√	Type of study designs used	Prospective (cohort, case-cohort or "nested case control") population-based studies
√	Study population	Approximately general populations (i.e., did not select participants on the basis of confirmed pre-existing medical conditions such as hypertension, cardiovascular disease, liver disease, or chronic kidney disease at baseline).
Reporting of search strategy should include		
√	Qualifications of searchers	Setor Kunutsor, MD PhD; Hassan Khan, MD PhD
√	Search strategy, including time period included in the synthesis and keywords	Time period: from inception of MEDLINE, EMBASE, Web of Science to November 2015. **Search strategy:** 1 (Gamma glutamyltransferase"[MeSH] OR "gamma glutamyltransferase"[All Fields]) 2 ("Heart failure"[MeSH] OR "heart pressure"[All Fields]) 3 ("humans"[MeSH Terms]) 4 (1 AND 2 AND 3)
√	Databases and registries searched	MEDLINE, EMBASE, and Web of Science
√	Search software used, name and version, including special features	Ovid was used to search EMBASE Reference Manager used to manage references
√	Use of hand searching	We searched bibliographies of retrieved papers
√	List of citations located and those excluded, including justifications	Details of the literature search process are outlined in the flow chart. The citation list for excluded studies is available upon request.
√	Method of addressing articles published in languages other than English	We placed no restrictions on language
√	Method of handling abstracts and unpublished studies	None found
√	Description of any contact with authors	None
Reporting of methods should include		
√	Description of relevance or appropriateness of studies assembled for assessing the hypothesis to be tested	Detailed inclusion and exclusion criteria are described in the Methods section.
√	Rationale for the selection and coding of data	Data extracted from each of the studies were relevant to the population characteristics, study design, exposure, outcome, and possible effect modifiers of the association.
√	Assessment of confounding	We assessed confounding by ranking individual studies on the basis of different adjustment levels.
√	Assessment of study quality, including blinding of quality assessors; stratification or regression	Not applicable

	on possible predictors of study results	
√	Assessment of heterogeneity	Heterogeneity of the studies was explored with I^2 statistic that provides the relative amount of variance of the summary effect due to the between-study heterogeneity.
√	Description of statistical methods in sufficient detail to be replicated	Description of methods of meta-analyses and sensitivity analyses are detailed in the methods. We performed random effects meta-analysis (with a fixed effects model as a subsidiary analysis) with Stata 13.
√	Provision of appropriate tables and graphics	Table 1 and Figure 3
Reporting of results should include		
√	Graph summarizing individual study estimates and overall estimate	Figure 3
√	Table giving descriptive information for each study included	Supplementary Material 9
√	Results of sensitivity testing	Sensitivity analysis was conducted to assess the influence of each individual study by omitting one study at a time and calculating a pooled estimate for the remainder of the studies. Results section
√	Indication of statistical uncertainty of findings	95% confidence intervals were presented with all summary estimates, I^2 values and results of sensitivity analyses
Reporting of discussion should include		
√	Quantitative assessment of bias	Not applicable
√	Justification for exclusion	All studies were excluded based on the pre-defined inclusion criteria in methods section.
√	Assessment of quality of included studies	Brief discussion included in 'Methods' section
Reporting of conclusions should include		
√	Consideration of alternative explanations for observed results	Discussed in the context of the results.
√	Generalization of the conclusions	Discussed in the context of the results.
√	Guidelines for future research	Assessment of the causal relevance of GGT to risk of HF
√	Disclosure of funding source	No separate funding was necessary for the undertaking of this systematic review.

Supplementary Material 5: Literature search strategy

Relevant studies, published before November 29, 2015 (date last searched), were identified through electronic searches not limited to the English language using MEDLINE, EMBASE, and the Science Citation Index databases. Electronic searches were supplemented by scanning reference lists of articles identified for all relevant studies (including review articles), by hand searching of relevant journals and by correspondence with study investigators. The computer-based searches combined search terms related to gamma-glutamyltransferase and hypertension without language restriction.

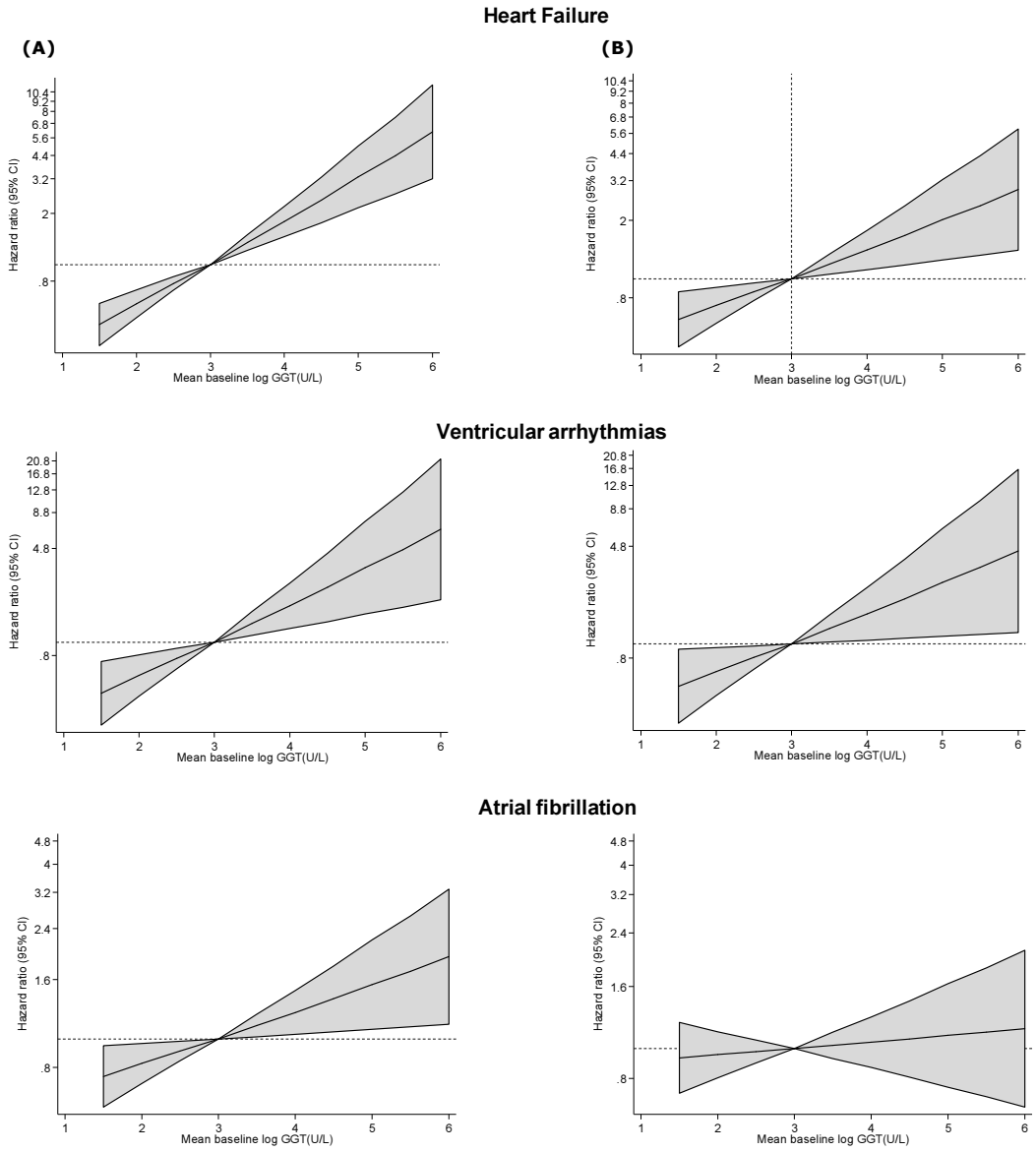
(i) MEDLINE strategy to identify relevant exposures:
(“Gamma glutamyltransferase”[MeSH] OR "gamma glutamyltransferase"[All Fields] OR "Gamma glutamyltranspeptidase"[MeSH] OR "gamma glutamyltranspeptidase"[All Fields])

(ii) MEDLINE strategy to identify relevant outcomes:
("Heart failure"[MeSH] OR “heart failure”[All Fields] OR "Ventricular dysfunction"[MeSH] OR “ventricular dysfunction”[All Fields])

(iii) MEDLINE strategy to identify relevant population:
("humans"[MeSH Terms])

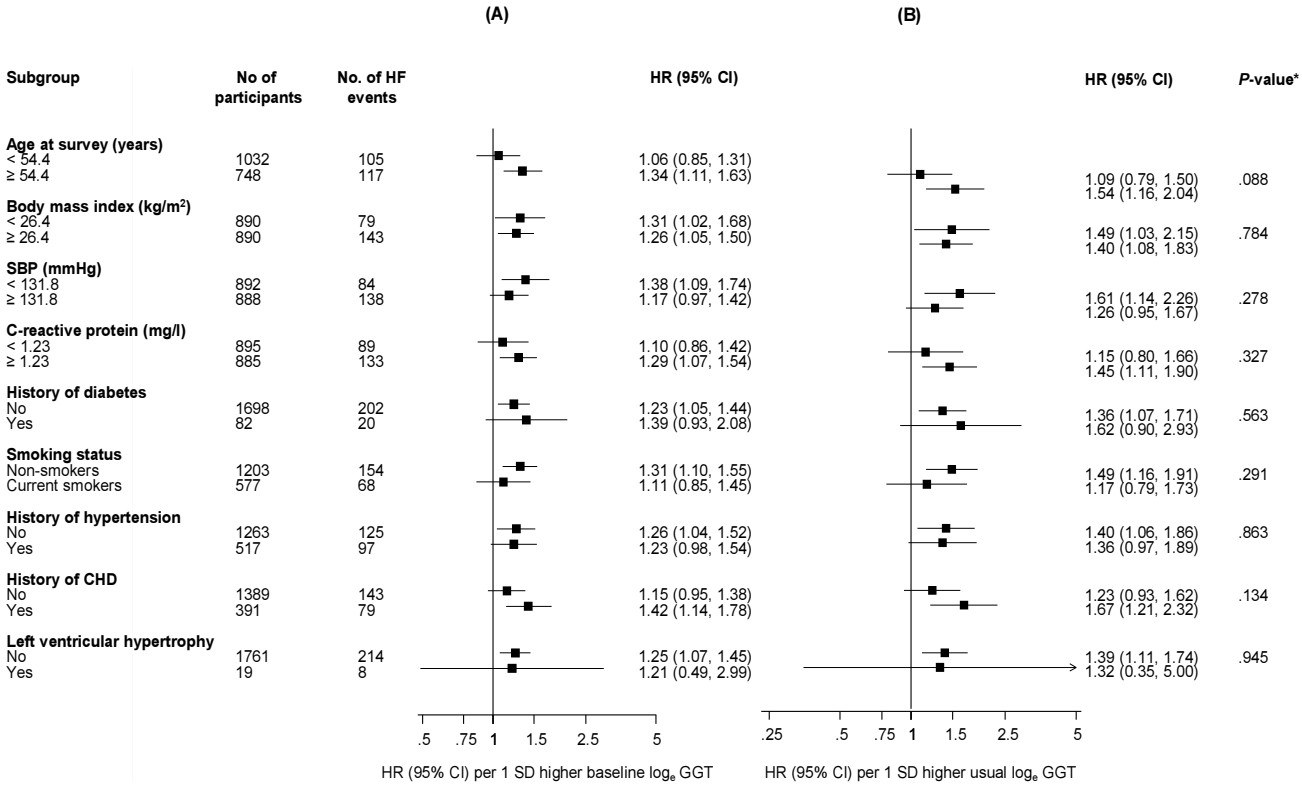
Parts i, ii and iii were combined using ‘AND’ to search MEDLINE. Each part was specifically translated for searching alternative databases.

Supplementary Material 6: Hazard ratios for heart failure, ventricular arrhythmias, and atrial fibrillation using multivariate-adjusted fractional polynomials



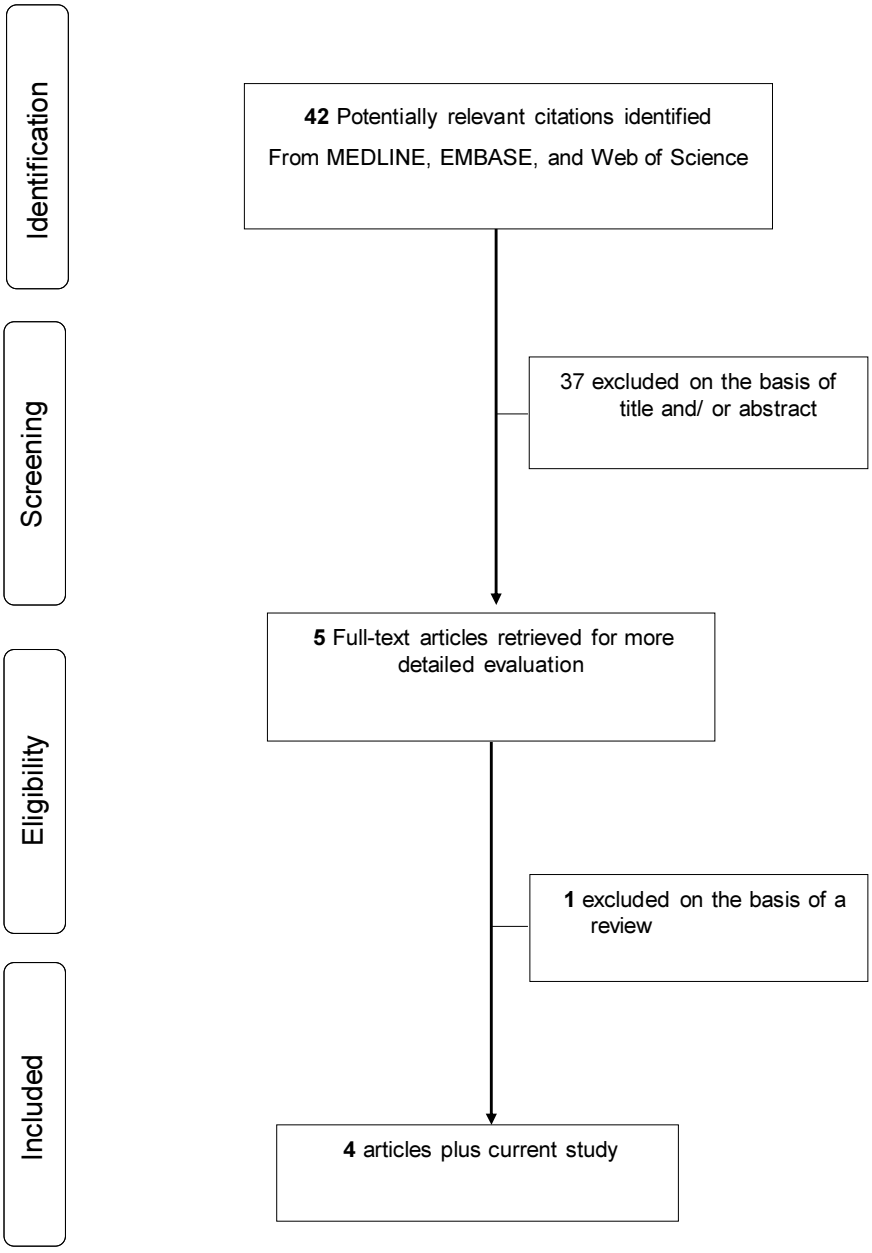
A, adjusted for age; **B**, adjusted for age, body mass index, systolic blood pressure, prevalent coronary heart disease, smoking status, history of diabetes, left ventricular hypertrophy, and use of medications (antihypertensive agents and lipid-lowering drugs); GGT, gamma glutamyltransferase

Supplementary Material 7: Hazard ratios for baseline and usual $\log_e$ GGT values and heart failure risk by several participant level characteristics



Hazard ratios (HRs) were adjusted for age, body mass index, systolic blood pressure, prevalent coronary heart disease, smoking status, history of diabetes, left ventricular hypertrophy, and use of medications (antihypertensive agents and lipid-lowering drugs); **A**, HRs per 1 standard deviation (SD) higher baseline $\log_e$ GGT values; **B**, HRs per 1 SD higher usual $\log_e$ GGT values; CHD, coronary heart disease; CI, confidence interval; GGT, gamma-glutamyltransferase; HF, heart failure; SBP, systolic blood pressure; *, *P*-value for interaction

Supplementary Material 8: Selection of studies included in the meta-analysis



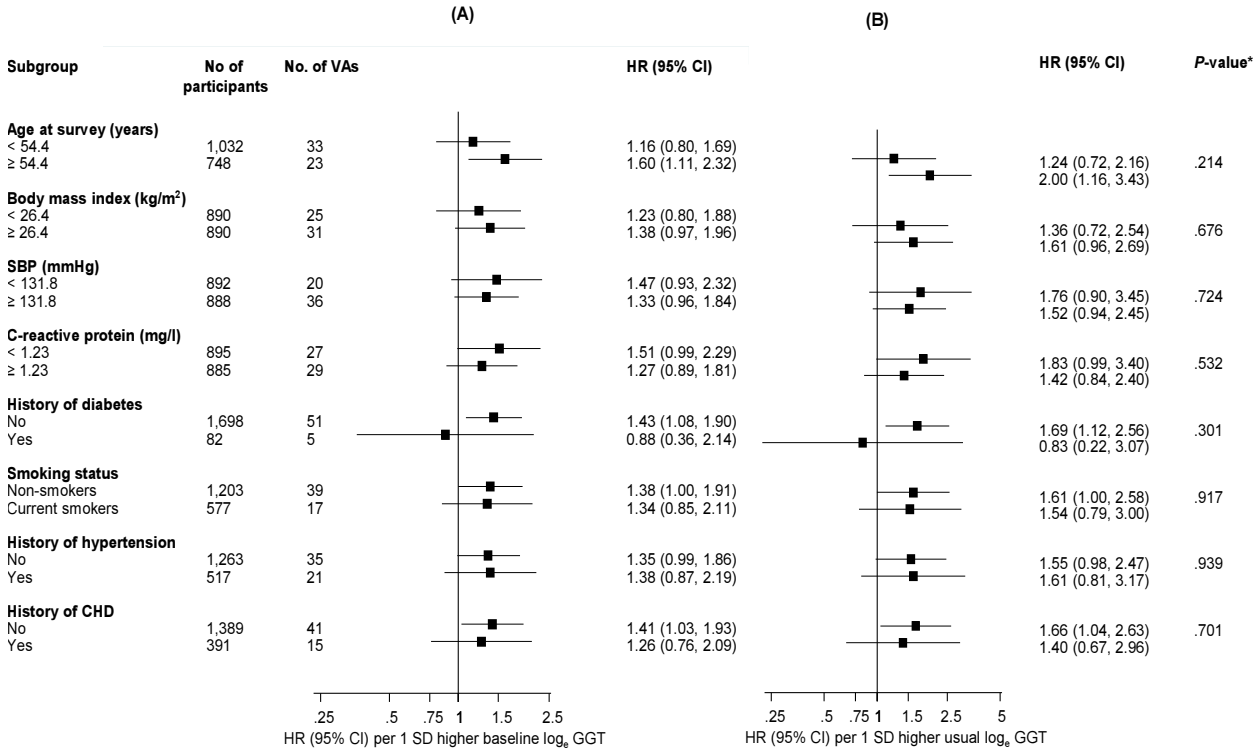
Supplementary Material 9: Prospective studies of gamma-glutamyltransferase and incident heart failure

Author, year of publication	Study	Location	Population source	Year of baseline survey	Age range at baseline (yrs.)	Male (%)	Mean follow-up (yrs.)	No. of cases	No. of participants	Covariates adjusted for
Ruttmann, 2005	VHM&PP	Austria	Population register	1985-2001	>/=19	45.6	17	162	163,944	**Age, BMI, SBP, cholesterol, TGs, glucose, smoking, work status, year of examination**
Dhingra, 2010	FHS Offspring	USA	FHS register	1948	44†	48.3	23.6	188	3,544	**Age, sex, BMI, DM, smoking, SBP, treatment for hypertension, alcohol intake, total/HDL-cholesterol ratio, valve disease, history of MI, AST, ALT, CRP**
Wannamethee, 2012	BRHS	UK	GP register	1978-1980	60-79	100.0	9	168	3,494	**Age, BMI, smoking, social class, alcohol intake, prevalent stroke, DM, AF, LVH, antihypertensive drugs, FEV1, SBP, cholesterol, CRP, vWF, leptin, NT-proBNP, HOMA-IR, AST/ALT**
Wang, 2013	FINRISK	Finland	Population register	1982 / 1987 / 1992 / 1997 / 2002	25-74	48.2	14.5	1,081	38,079	**Age, study area, study year, smoking, education, alcohol consumption, PA, history of valvular disease, BMI, SBP, cholesterol at baseline, MI**
Current Study	KIHD	Finland	Population register	1984-1989	42-61	100	22.0	222	1,780	**Age, BMI, SBP, prevalent coronary heart disease, smoking status, history of diabetes, LVH, use of medications (antihypertensive agents and lipid-lowering drugs), alcohol consumption, resting heart rate, TGs, total cholesterol, HDL-cholesterol, CRP, and incident CHD as a time-varying covariate**
	Total							**1,821**	**210,841**	

*Range of follow-up. †Mean age at baseline

Abbreviations: AF, atrial fibrillation; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BRHS, British Regional Heart Study; CHD, coronary heart disease; CRP, C-reactive protein; DM, diabetes mellitus; FEV1, (forced expiratory volume in 1 second; FHS, Framingham Heart Study; HDL, high-density lipoprotein; HOMA-IR, homeostasis model assessment for insulin resistance; KIHD, Kuopio Ischaemic Heart Disease Study; LVH, left ventricular hypertrophy; MI, myocardial infarction; NT-proBNP, N terminal pro brain natriuretic peptide; PA, physical activity; SBP, systolic blood pressure; TG, triglyceride; VHM&PP, The Vorarlberg Health Monitoring and Promotion Program; vWF, von Willebrand factor

Supplementary Material 10: Hazard ratios for baseline and usual log_e GGT values and ventricular arrhythmias risk by several participant level characteristics



Hazard ratios were adjusted for age, body mass index, systolic blood pressure, prevalent coronary heart disease, smoking status, history of diabetes, left ventricular hypertrophy, and use of medications (antihypertensive agents and lipid-lowering drugs); **A**, HRs per 1 standard deviation (SD) higher baseline log_e GGT values; **B**, HRs per 1 SD higher usual log_e GGT values; CHD, coronary heart disease; CI, confidence interval; GGT, gamma-glutamyltransferase; HR, hazard ratio; SD, standard deviation; VAs, ventricular arrhythmias; *, *P*-value for interaction